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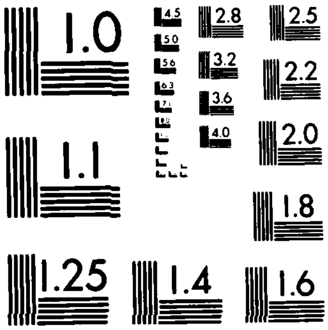

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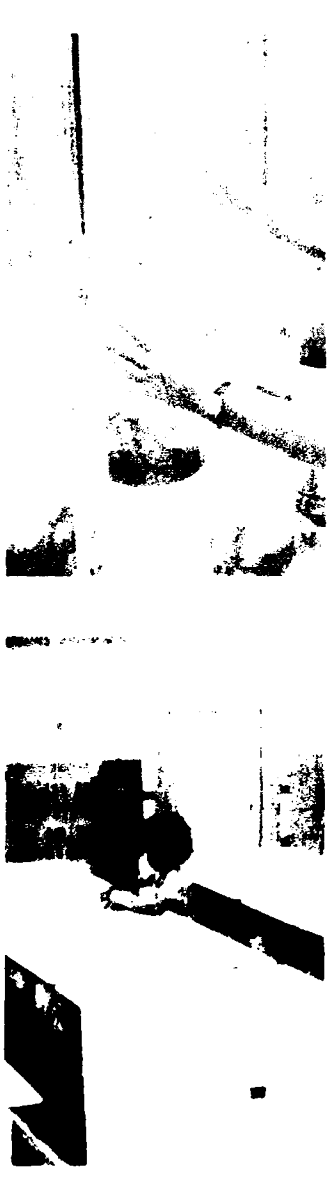

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CULTURE METHODOLOGY FOR EXPERIMENTAL INVESTIGATIONS INVOLVING ROOTED SUBMERSED AQUATIC PLANTS

by

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Recent information on the relative roles of sediment and water as nutrient sources for rooted submersed freshwater macrophytes has facilitated the development of a methodology for culturing these plants. The use of natural sediment as the source of nitrogen, phosphorus, and micronutrients, coupled with the omission of these nutrients from culture solution, largely prevents the occurrence of algal blooms and, for many purposes, obviates the use of elaborate axenic culturing techniques.

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ABSTRACT (Continued).

Contemporary information on the growth requirements of submersed macrophytes is reviewed in relation to the provision of conditions suitable for the laboratory culture of these plants. Sediment substrate requirements are considered in relation to the role of sediment as a nutrient source. Two culture solution formulations are provided along with procedures for solution preparation. Procedures for establishing and maintaining cultures are also provided. The information presented is intended to be of assistance in the establishment and maintenance of submersed macrophyte cultures for experimental research.

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Preface

The culture methodology presented in this report is based on contemporary information on the growth requirements of rooted submersed aquatic plants and incorporates pertinent findings from a number of investigations conducted in the US Army Engineer Waterways Experiment Station (WES) Enviornmental Laboratory (EL) between 1978 and 1984. Funding for these investigations was provided by the Office, Chief of Engineers (OCE), through the Aquatic Plant Control Research Program (APCRP) and the Environmental and Water Quality Operational Studies (EWQOS) Program. Technical Monitors for OCE during this study were Mr. E. Carl Brown for APCRP and Mr. Earl Eiker, Dr. John Bushman, and Mr. James L. Gottesman for EWQOS.

These investigations were performed under the general supervision of Dr. John Harrison, Chief, EL, and the direct supervision of Mr. Donald L. Robey, Chief, Ecosystem Research and Simulation Division (ERSD), WES, and Dr. Tom L. Hart, Chief, Aquatic Processes and Effects Group (APEG), ERSD. Authors of this report were Mr. R. Michael Smart and Dr. John W. Barko, APEG. Technical assistance was provided by Mr. Lee Ferguson, Ms. Susan Hennington, Ms. Dwillette McFarland, and Ms. Ramona Warren. Program Managers were Mr. J. Lewis Decell (APCRP) and Dr. Jerome L. Mahloch (EWQOS).

Commander and Director of WES during publication of this report was COL Robert C. Lee, CE. Technical Director was Mr. F. R. Brown.

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Contents

CULTURE METHODOLOGY FOR EXPERIMENTAL INVESTIGATIONS INVOLVING ROOTED SUBMERSED AQUATIC PLANTS

Introduction

Background

1. One of the major problems of growing submersed aquatic plants in the laboratory is that of providing adequate nutrition while minimizing the growth of phytoplankton and attached algae, which, if uncontrolled, prohibits the development of macrophyte cultures (Gerloff and Krombholz 1966; Mulligan and Baranowski 1969; Ryan, Reimer, and Toth 1972; Mulligan, Baranowski, and Johnson 1976). Unfortunately, algae-free cultures are difficult to obtain (Wetzel and McGregor 1968) and require considerable care to prevent contamination over long periods of time (Denny 1980). In spite of the above problems, early efforts to culture submersed macrophytes were apparently influenced by the then widespread belief that most nutrients were obtained by shoot uptake from the water (see reviews by Bourn (1932) and Sculthorpe (1967)). Early culture media for submersed macrophytes were, therefore, understandably based almost exclusively on those developed for macrophytic algae (Pringsheim and Pringsheim 1962; Forsberg 1965; Imahori and Iwasa 1965) or hydroponic culture of terrestrial plants (Hoagland and Arnon 1938). Complete nutrient solutions based on algal media (e.g. Wetzel and Manny 1972) clearly favor the growth of phytoplankton and attached algae while those based on hydroponic media (Gaudet 1963; Gerloff and Krombholz 1966; Stanley 1970; Basiouny, Garrard, and Haller 1977), in addition to promoting algal growth, bear little chemical resemblance to natural waters.

2. Recently, however, the earlier consideration of roots as merely organs of attachment (Brown 1913; Den Hartog and Segal 1964) has been questioned due to the morphological and functional similarities between roots of submersed macrophytes and those of terrestrial plants (Bristow 1975). Only within the past decade has the importance of nutrient uptake by roots of submersed macrophytes been fully realized (see review by Denny (1980)). This advance in our understanding of macrophyte nutrition facilitates the development of culture methodology employing solutions formulated specifically for growing submersed macrophytes on natural sediment.

Objective and scope

3. The objective of this report is to incorporate contemporary

information on the growth requirements of submersed macrophytes in the development of methodology for the laboratory culture and study of rooted submersed freshwater macrophytes. The information provided is intended to be of assistance in establishing and maintaining cultures of plants for experimental research, including physiological and ecological investigations, preliminary herbicide evaluations, and screening of microbial pathogens or herbivorous insects as potential weed control agents. Plant cultures may also be useful in providing mass quantities of physiologically or genetically similar plant tissues for subsequent observation or experimentation.

Culture Requirements

Nutrient sources

4. Although specific sites of nutrient uptake by submersed macrophytes may vary in relation to environmental (sediment/water) nutrient availability (Denny 1972; Patterson and Brown 1979; Barko 1982; Carignan 1982; Waisel, Agami, and Shapira 1982), it is now generally accepted that rooted submersed macrophytes can fulfill their phosphorus requirements by uptake from sediments (Bristow and Whitcombe 1971; DeMarte and Hartman 1974; Best and Mantai 1978; Bole and Allan 1978; Carignan and Kalff 1979, 1980; Welsh and Denny 1979a; Barko and Smart 1980, 1981a; Huebert and Gorham 1983). Recent studies have likewise demonstrated significant mobilization of nitrogen from sediments (Toetz 1974; Nicholls and Keeney 1976a, b; Best and Mantai 1978; Barko and Smart 1981a; Barko 1982; Huebert and Gorham 1983). Moreover, additions of nitrogen to solution, sediment, or both have not resulted in increased plant growth relative to unfertilized controls (Barko 1983; Barko and Smart 1983). Therefore, sufficient nitrogen and phosphorus to support adequate plant growth can usually be obtained from sediment in the absence of solution nitrogen or phosphorus.

5. While nitrogen and phosphorus are primarily acquired by root uptake from sediment, potassium appears, at least in some species, to be absorbed primarily from solution (Barko and Smart 1981a; Barko 1982). Although some sediments may provide sufficient potassium for moderate growth of submersed macrophytes, addition of potassium to solution usually stimulates growth and increases tissue potassium concentrations (Barko 1982; Huebert and Gorham 1983). To ensure optimal growth conditions, potassium should be included in culture solutions.

6. Calcium may be mobilized from sediment by some submersed macrophytes (DeMarte and Hartman 1974); however, *Potamogeton pectinatus* L. failed to grow in the absence of solution calcium (Huebert and Gorham 1983), and growth of *Myriophyllum spicatum* L. was reduced in solutions low in calcium (Barko 1982). The important role of calcium as a component of the carbonate system, in addition to its apparent involvement in photosynthetic bicarbonate utilization (Lowenhaupt 1956; Lucas and Dainty 1977), require that calcium be included in culture solution.

7. The relative importance of sediment and water as sources of magnesium, sodium, chloride, and sulfur for rooted submersed macrophytes is unclear. Reduced growth of *P. pectinatus* in solutions lacking magnesium (Huebert and Gorham 1983) suggests that magnesium may be acquired primarily from solution. Active foliar uptake of sulfate (SO_4) and chloride has been demonstrated in several rooted submersed macrophyte species (see review by Denny (1980)), indicating that these ions can be readily acquired from solution. However, omission of sulfate from solution did not affect the growth of *P. pectinatus* (Huebert and Gorham 1983), suggesting that sulfur can be obtained from sediment in the absence of solution sulfate. Similarly, both shoot and root uptake of sodium and chloride have been demonstrated in several species (Waisel, Agami, and Shapira 1982). Moreover, Shepherd and Bowling (1973) demonstrated active sodium and chloride uptake by roots of *Potamogeton natans*. Therefore, while magnesium may be required in culture solutions, plant demand for sodium, chloride, and sulfur apparently may be fulfilled by either root uptake from sediment or shoot uptake from solution. We generally include sodium, sulfate, and chloride in culture solutions because of their widespread occurrence in fairly high concentration in natural waters and as a convenient means of adding required amounts of bicarbonate, magnesium, and calcium, respectively.

8. Rooted aquatic plants are presumably able to satisfy their requirements for micronutrients by uptake from sediment (Huebert and Gorham 1983). Evidence for root uptake of iron (DeMarte and Hartman 1974; Gentner 1977; Basiouny, Haller, and Garrard 1977; Barko and Smart 1983), manganese (Barko and Smart 1983), copper (Welsh and Denny 1979b, 1980; Cushing and Thomas 1980), and zinc (Cushing and Thomas 1980) has been presented for some submersed freshwater macrophytes. The relative importance of root/shoot uptake of boron and molybdenum is unknown. We have observed no adverse effects attributable to the omission of boron, molybdenum, or other micronutrients from culture solutions,

and additions of boron, iron, manganese, zinc, and molybdenum to solution have not resulted in increased growth of *H. verticillata* (unpublished data) or *P. pectinatus* (Huebert and Gorham 1983).

Carbon supply and pH

9. Inorganic carbon sources in nature include dissolved inorganic carbon (DIC), CO_2 supplied by air/water exchange, and heterotrophic production of CO_2 by sediment and water-column respiration. Dissolved inorganic carbon should be included in culture solutions due to the importance of carbonate equilibria on chemical and biological processes in fresh waters (Stumm and Morgan 1981) and the ability of many submersed macrophyte species to utilize bicarbonate, in addition to free CO_2, in photosynthesis (Raven 1970). Aeration should be provided to enhance the air/water exchange of CO_2; however, aeration alone cannot substitute for bicarbonate as a carbon source. We have been unable to obtain significant growth of either *H. verticillata* or *M. spicatum* in the absence of added bicarbonate in spite of vigorous aeration at ambient CO_2 levels.

10. Solution pH will be controlled by the levels of alkalinity and DIC. The use of organic buffers to artificially control pH is generally unnecessary and should be avoided due to possible toxicity of some buffers (Stanley 1970). While many species are adaptable to wide ranges in alkalinity, pH, and DIC, some species, generally those restricted to low pH/low alkalinity waters (Moyle 1945; Spence 1967; Hutchinson 1970; Seddon 1972; Hellquist 1980; Kadono 1982), may be unable to efficiently utilize bicarbonate in photosynthesis (Steemann Nielsen 1947; Kadono 1980; Allen and Spence 1981). These acidophilic species may require aeration with CO_2-enriched air to achieve normal growth and metabolism. Augmenting the airstream CO_2 supply decreases pH and shifts carbonate equilibria to increase the proportion of free CO_2 ($CO_2 + H_2CO_3$) in solution.

Facilities

11. Vessels suitable for the culture of submersed macrophytes are available in a variety of inert materials. We have used white fiberglass-reinforced polyester tanks for a number of years and found them to be durable yet sufficiently lightweight for portability. Areal dimensions (1 to 2 m on a side in our laboratory) may vary depending on application and space availability, but the depth should approximate 1 m to accomodate the vertical growth of the cultured species. Larger or deeper vessels may be used but these are more costly, less convenient during planting and harvesting operations, and less portable. Deeper vessels may also produce significant shadowing problems under natural

lighting due to shading by the sidewalls. Culture vessels should be covered with a transparent material such as lucite to prevent the entry of dust and other airborne contaminants.

12. Aeration and mixing can be provided by airlifts constructed of plastic (polyvinyl chloride) pipe or other chemically inert material and fitted with air diffusers of the type used for aquarium aeration. Air may be provided by aquarium air pumps, but larger scale facilities may require a compressor and storage tank. To minimize evaporation from the culture solution, compressed air can be passed through a humidification column prior to introduction to the culture vessel.

13. Photosynthetically active radiation (PAR) levels between 300 and 1000 $\mu E\ m^{-2} sec^{-1}$ are suitable for the culture of most strictly submersed species (Bowes, et al. 1977; Titus and Adams 1979; Barko and Smart 1981b; Barko, Hardin, and Matthews 1982); however, floating-leaved species may benefit from higher levels (Barko, Hardin, and Matthews 1982). Room temperatures (20^{o}-$25^{o}C$) are adequate for most species; however, some of the more subtropically occurring, exotic species (e.g. *H. verticillata*) may benefit from higher temperatures (Van, Haller, and Garrard 1978; Bowes, Holaday, and Haller 1979; Barko and Smart 1981b). Neutral density shade fabric may be used, if necessary, to reduce light intensity and solar heating in outdoor/glasshouse cultures. Heating can be simply and inexpensively provided with aquarium-type immersion heaters. However, in many cases it may be more convenient to employ liquid circulators, which provide both heating and cooling, while continuously circulating the culture solution (Barko and Smart 1981b).

Culture Development

Culture solutions

14. Culture solutions presented here were devised on the assumption that nitrogen, phosphorus, and micronutrients are obtained primarily by root uptake from sediments. The use of natural sediments as a nutrient substrate coupled with the omission of nitrogen and phosphorus from culture solutions largely prevent the occurrence of algal blooms in plant cultures, obviate the use of elaborate techniques for exenic culturing, and allow the use of culture solutions chemically more similar to natural waters than to artificial growth media. However, in some cases it may be necessary or desirable to axenically culture

plants on totally artificial media (Wetzel and McGregor 1968; Klaine and Ward 1981). Complete nutrient media which may be appropriate for axenic cultures are provided in Gerloff and Krombholz (1966), Bristow and Whitcombe (1971), and Wetzel and Manny (1972).

15. In many investigations, and for routine culture, it is desirable to provide a simple, yet near-optimal solution composition. For routine usage the general culture solution shown in Table 1 is easily formulated. Alternatively, some investigations may require the use of higher alkalinity solutions or solutions of more balanced composition (with respect to major cation and bicarbonate levels) to more closely approximate natural conditions. An important consideration relevant to the use of solutions high in calcium and bicarbonate is the increased likelihood of $CaCO_3$ precipitation resulting in changes in solution composition. An additional consideration is the resultant increased ash content of submersed macrophyts shoots due to $CaCO_3$ precipitation on leaf surfaces. We have attributed a slight reduction in growth of *M. spicatum* at elevated $Ca(HCO_3)_2$ levels to the development of a heavy precipitate on the leaves (unpublished data). Both of these problems may be ameliorated and plant growth increased by augmenting the airstream CO_2 concentration.

16. One of the problems of formulating moderate to high alkalinity solutions is the low solubility of calcium and magnesium carbonates and the unavailability of bicarbonate salts of these cations. Provision of carbon solely as sodium and potassium bicarbonates avoids the problem of solubility, but results in unnaturally high monovalent cation concentrations and atypical ratios among the major cations. For these reasons we prefer adding bicarbonate by simulating the natural weathering process of acid dissolution of $CaCO_3$. We have accomplished complete solubility of added $CaCO_3$ by administering CO_2 to the solution prior to adding the required $CaCO_3$. Dissolution is fairly rapid, but prolonged aeration with ambient air is required to subsequently equilibrate pCO_2 with atmospheric levels. This technique can be used to prepare solutions of various alkalinity and DIC levels such as the solution presented in Table 2 employing cation and bicarbonate proportions based on data obtained from alkaline lakes (Hutchinson 1957).

17. When preparing large quantities of solution it is desirable to measure alkalinity and pH as well as the concentrations of major components such as calcium and DIC to ensure that complete solubilization has occurred and that no gross errors or omissions of reagents have occurred. Equilibration

of the carbonate system can be verified from pH, DIC, and alkalinity (Stumm and Morgan 1981). Levels of DIC can be determined with an infrared gas analyzer or other carbon analyzer and calcium concentrations by atomic absorption spectrophotometry. However, in cases where these instruments are not readily available, and for rapid routine monitoring, ionic strength (an indicator of ionic composition) can be checked by measuring conductivity. We have used the relationship between conductance and ionic strength (Snoeyink and Jenkins 1980) to check the gross chemical composition of solutions spanning a sixfold range in alkalinity and major cation concentrations (Figure 1). Due to the strong influence of divalent cations on ionic strength, incomplete solubilization or precipitation of $CaCO_3$ can be readily detected. For example, failure to dissolve 10 mg ℓ^{-1} calcium as $CaCO_3$ will result in a reduction in conductivity (25^oC) of approximately 45 μS cm^{-1}, while a similar loss due to precipitation of $CaCO_3$ will reduce conductivity approximately 35 μS cm^{-1}.

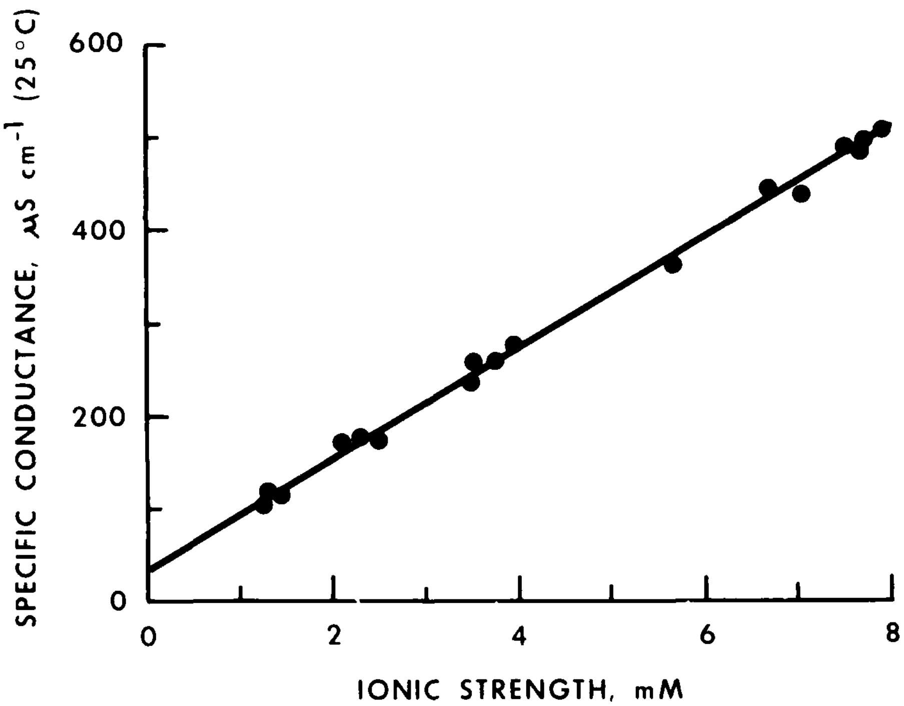

Figure 1. Relationship between specific conductance and ionic strength for 17 experimental solutions of various cation and bicarbonate concentrations

Sediment substrate

18. We have obtained maximum yields of a variety of submersed aquatic plant species on fine-textured lake sediments with an organic content of less

than 15 percent dry weight. Sediments containing higher levels of refractory organic matter or a substantial (>75 percent) sand-sized fraction are generally poor substrates for submersed aquatic plants (Barko and Smart, subm.) However, on fine-textured, mineral sediments we frequently obtain yields equivalent to 1200 g dry wt m^{-2} sediment surface area.

19. It should be noted that commercially available potting soils are generally unsuitable substrates for submersed aquatic plant growth. Cultures initiated on several types of organic potting soils exhibited greatly reduced growth, chlorosis, or death (personal observation), perhaps due to the anaerobic formation of inhibitory organic compounds (Harper and Lynch 1982; Barko and Smart 1983). In addition many potting soil mixtures contain low-density components (perlite, vermiculite, or peat), which are buoyant, resulting in undesirable turbidity and, ultimately, algal blooms in the culture solution.

20. Peat-sand or muck-sand mixtures have been frequently used for culturing and experimental studies of rooted submersed macrophytes in flow-through systems (Steward and Center 1979; Langeland and Sutton 1980; Sutton, Littell, and Langeland 1980; Westerdahl and Hall 1983); however, the use of these relatively infertile substrates may result in nutrient limitation (Langeland, Sutton, and Canfield 1983; Hall, Westerdahl, and Stewart 1984). Plant growth has also been shown to be considerably reduced on peat-sand relative to that obtained on fine-textured lake sediment (Hall, Westerdahl, and Stewart 1984). These problems might be alleviated by addition of nutrients to the water or substrate; however, nutrient enrichment may result in excessive growth of phytoplankton and attached algae (Mulligan and Baranowski 1969; Ryan, Reimer, and Toth 1972; Mulligan, Baranowski, and Johnson 1976).

21. Sediment for plant culture can be obtained by dredging and should be allocated to planting containers several days prior to use to allow for settling. Sediment containers providing a depth of 10 to 20 cm and a volume of 1 to 4 ℓ are suitable for most species. Approximately 2 cm should be allowed for placement of a layer of coarse silica sand over the sediment to minimize physical exchanges between the sediment and the culture solution (Hynes and Grieb 1970).

Plant propagation

22. We have used different methods of plant propagation depending upon the growth form of the species selected for culture. Many species can be rooted from apical cuttings. Fresh apical cuttings 10 to 15 cm in length are taken from healthy, nonflowering stems and inserted to a depth of about 5 cm into the

sediment. Root formation generally begins within 1 week. Stocks of plant species amenable to this method of propagation can be rapidly expanded by repeated subculturing.

23. Some species produce underground vegetative organs (tubers/rhizomes) (Sculthorpe 1967; Van, Haller, and Garrard 1978) or specialized apices (turions) (Sculthorpe 1967; Sastroutomo 1980, 1981) which can be obtained directly from the field or from biological suppliers. Vegetative propagules of the above types can usually be stored in a refrigerator between layers of moist toweling for several months until needed. Species which readily reproduce by stolons or runners are best planted as intact plants (Sculthorpe 1967). Many species can be propagated from seed in addition to the above methods. Additional information relevant to propagation has been reviewed by Sculthorpe (1967).

Culture Maintenance

Subculturing

24. After prolonged periods of growth, cultures of submersed macrophytes lose viability, either due to the onset of senescence or to the depletion of sediment or water resources. In order to maintain viable cultures over long periods, the investigator must periodically initiate subcultures from secondary cuttings. The frequency of subculturing will depend on rates of growth and phenological development; therefore, cultures maintained at higher temperatures will require more frequent subculturing to prevent deterioration (Barko and Smart 1981b). Some species (notably *M. spicatum*) are prone to flowering and subsequent senescence soon after developing a canopy at the water surface (Grace and Wetzel 1978; Barko and Smart 1981b). The investigator should observe plant cultures frequently and initiate subcultures prior to the onset of flowering since flowering apices generally exhibit a greatly reduced ability to form roots.

25. Subcultures should be initiated on fresh sediment, as we have repeatedly observed diminished growth of several species on previously planted sediments. Whether this diminished growth is due to nutrient depletion, accumulation of toxins, or some other mechanism is unclear and is the subject of current investigation.

Algal control

26. During short-term experiments (6 to 8 weeks), the development of

algal populations in solutions lacking nitrogen and phosphorus is usually minor. However, in mass cultures subjected to repeated clipping, or in extended experiments involving some degree of tissue senescence or damage, algal populations may reach undesirable levels. Phytoplankton can be largely controlled through the use of diatomaceous earth filters of the type commonly used for aquariums. This method of control is favored as it minimizes disturbance of the plants, does not require toxic chemicals, minimizes chemical changes in the solution, and effectively removes nitrogen and phosphorus incorporated into the algal cells, thus reducing the probability and severity of subsequent algal blooms. The use of algicides such as $CuSO_4$ should be avoided as these may be toxic to aquatic plants even in low concentrations (Ryan and Riemer 1975).

Maintenance of solution composition

27. One of the major differences between a culture of submersed macrophytes and a field population is a greatly reduced rate of water-column and sediment respiration in the former. For this reason, carbon supplied as DIC to macrophyte cultures may be inadequate to support photosynthetic carbon requirements. While aeration provides a major source of inorganic carbon, we have observed significant reductions in DIC and subsequent increases in pH due to plant uptake of DIC. Reduction in DIC may also result from precipitation of $CaCO_3$. Increasing the air/water exchange of CO_2 by increasing the aeration rate or by augmenting the CO_2 concentration in the air stream should ameliorate the above problems. Changes in solution composition due to reduction in DIC can also be lessened by using low calcium, low alkalinity solutions such as the general culture solution described here; however, in this case it should be realized that most of the carbon required for plant growth must be provided through aeration.

28. Another major difference between a culture of submersed plants and a field population is a reduced rate of water exchange (particularly in static cultures). Changes in chemical composition of culture solutions are ultimately dependent on the ratio of solution volume to plant biomass. In productive systems in the temperate zone, submersed macrophytes may attain a biomass of 500 g dry wt m^{-2} (Westlake 1975) in a water column of about 2 m in depth (2000 ℓ m^{-2}). These figures provide a minimal solution volume:biomass ratio of 4 ℓ g^{-1} (ignoring water exchange). However, for experimental cultures, we recommend a solution volume:biomass ratio of 10 or higher to avoid substantial changes in solution composition. The use of the largest practical culture

vessels with sediment containers occupying ≤10 percent of the bottom surface area minimizes crowding of plants and sediment-water exchange of nitrogen and phosphorus, and generally provides a sufficiently high solution:biomass ratio. Low volume systems may require continuous flow (Westerdahl and Hall 1983) or partial solution changes (Barko 1982) to avoid substantial changes in solution composition. For routine culturing, solution volume:biomass ratios can be increased and sediment containers can occupy 50 percent of the bottom surface area of the culture vessel. In this case, changes in solution composition are minimized by the use of low calcium, low alkalinity solutions, and the frequent initiation of subcultures in fresh solution.

Experimental Use of Cultures

29. One objective of submersed macrophyte culturing may be direct experimentation (i.e. experimental manipulation of culture conditions). An important consideration in the experimental use of laboratory cultures is to provide near-natural conditions with respect to nonexperimental parameters while investigating macrophyte responses to specific, experimentally manipulated variables. Experimental investigations should be designed to maximize biomass increase, thereby ensuring that plant biomass, tissue nutrient concentrations, and physiological state of plant tissues reflect experimental conditions rather than those the plants were exposed to prior to investigation. Under optimal solution, substrate, light, and temperature conditions, sufficient plant growth can be obtained in 5 to 6 weeks. Longer experimental durations may be required in some cases, but some species exhibit a tendency to slough senescent tissues, contributing to the development of algal problems as well as obfuscating treatment-related differences in growth (Barko and Smart 1981b). An additional consideration in longer experiments is that plant growth may ultimately be limited by variables (light, space, carbon, nutrients, etc.) not chosen for study. Under such conditions treatment-related differences in plant growth may decrease over time as plant populations approach maximal biomass attainable under a given set of environmental conditions.

30. Another possible objective of plant culturing may be to provide mass quantities of physiologically or genetically similar plant tissues for subsequent observation or experimentation. These tissues, cultured under near-natural but controlled conditions, may be more suitable for physiological or

morphological study than those collected from temporally and spatially varying natural environments. An additional advantage to this approach is that plant tissues of a variety of species can be made available for laboratory study throughout the year or in areas remote from natural macrophyte populations.

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Table 1

Chemical Composition and Formulation of a General Purpose Culture Solution

Chemical Composition		Formulation	
Parameter	Concentration	Reagent	Quantity, mg ℓ^{-1}
Ca, mg ℓ^{-1}	25.0	$CaCl_2 \cdot 2H_2O$	91.7
Mg, mg ℓ^{-1}	6.8	$MgSO_4 \cdot 7H_2O$	69.0
Na, mg ℓ^{-1}	16.0	$NaHCO_3$	58.4
K, mg ℓ^{-1}	6.0	$KHCO_3$	15.4
DIC, mg ℓ^{-1}	10.2		
SO_4, mg ℓ^{-1}	26.9		
Cl, mg ℓ^{-1}	44.2		
Alkalinity, meq ℓ^{-1}	0.85		
Ionic strength, mM	3.9		
Conductivity, μS cm^{-1} ($25^{\circ}C$)	280.0		
pH (air equilibrium)	7.9		

Table 2

Chemical Composition and Formulation of an Ionically Balanced, Alkaline Solution*

Chemical Composition		Formulation	
Parameter	Concentration	Reagent	Quantity, mg ℓ^{-1}
Ca, mg ℓ^{-1}	60.0	$CaCl_2 \cdot 2H_2O$	91.6
Mg, mg ℓ^{-1}	10.1	$MgSO_4 \cdot 7H_2O$	102.3
Na, mg ℓ^{-1}	16.8	Na_2SO_4	52.0
K, mg ℓ^{-1}	6.1	K_2SO_4	13.6
DIC, mg ℓ^{-1}	21.0	$CaCO_3$**	87.5
SO_4, mg ℓ^{-1}	82.5		
Cl, mg ℓ^{-1}	44.2		
Alkalinity, meq ℓ^{-1}	1.75		
Ionic strength, mM	7.5		
Conductivity, μS cm^{-1} (25°C)	490.0		
pH (air equilibrium)	8.3		

* Cation and inorganic carbon proportions are based on data presented for alkaline lakes (Hutchinson 1957).

** Requires addition of CO_2 gas to achieve solubility. Note that approximately 50 percent of the final DIC concentration is derived from gaseous CO_2.

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